Supplemental information

Acquisition of the ability to assimilate mannitol by *Saccharomyces cerevisiae* through dysfunction of the general corepressor Tup1–Cyc8

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Supplementary Results

Isolation of Mtl+ strains. Fresh BY4742 cells (approximately 5×10^6 cells; OD_{600} of 1.0 corresponds to 0.7×10^7 cells/ml) were grown to log phase in liquid YPD medium and spread on SM plates. After 7 days of incubation, several visible colonies appeared on the plates (Fig. 1B). Two colonies were randomly picked and purified twice by streaking on SM plates. The resultant clones were grown in SM liquid medium for 2 days and stored as glycerol stocks (glycerol conc. 17%) at -80°C as MK3619 and MK3683 (Table 1).

BY4742 cells (approximately 10^7 cells) were harvested from YPG plates, suspended in sterilized distilled water (SDW), and spread on YPM plates. After 7 days of incubation, approximately 16 large colonies were formed. Eight large colonies were picked and purified as described above. Of those eight colonies, two exhibited no growth in SM liquid media, one colony exhibited the Flo+ phenotype in both SC and SM liquid media, and four colonies exhibited the Flo+ phenotype only in SC liquid medium and Flo- growth in SM liquid medium. The remaining colony, which exhibited Flo- growth in both SC and SM liquid media after 2 days of cultivation, was stored at -80°C as described above as MK4010 (Table 1) .

BY4742 cells (approximately 10^7 cells) were independently harvested from YPG plates, suspended in SDW, and spread on SM plates. After 7 days of incubation, more than 70 colonies were formed. Approximately 70 colonies were purified on SM plates as described above, and their growth in SM liquid medium after 2 days of cultivation was examined. Thirty-eight colonies exhibited growth in SM liquid medium and were stored at -80°C as described above. Of those 38 colonies, 14 strains (group 1) were Flo+

in both SC and SM liquid media, 8 (group 2) were Flo+ in SC but exhibited Flo- growth in SM medium, and 16 (group 3) exhibited Flo- growth in both SC and SM media. Five strains from group 1 (MK4421, MK4443, MK4446, MK4447, and MK4449; Table 1) and four strains from group 2 (MK4410, MK4412, MK4437, and MK4450; Table 1) were selected and further analyzed. The ability of the strains in group 3 to produce ethanol was examined as described in MATERIALS AND METHODS. Briefly, the strains were grown on YPM plates for 3 days, inoculated to OD_{600} of 0.1 in 50 ml YPM liquid medium in a 100 ml Erlenmeyer flask, and then grown at 30°C at 95 spm, collected, and suspended in SDW. The cells in the suspension were again inoculated to OD_{600} of 0.1 in 50 ml YPM liquid medium in a 100 ml Erlenmeyer flask, and then grown at 30°C at 95 spm for 3 days. Unexpectedly, only MK4416 and MK4456 produced significant amounts of ethanol (at 5,281 and 3,924 mg/l, respectively), whereas MK4424 and MK4461 produced only 179 and 180 mg/l ethanol, respectively; the other strains produced less than 10 mg/l ethanol. MK4416 and MK4456 (Table 1) were selected and further analyzed.

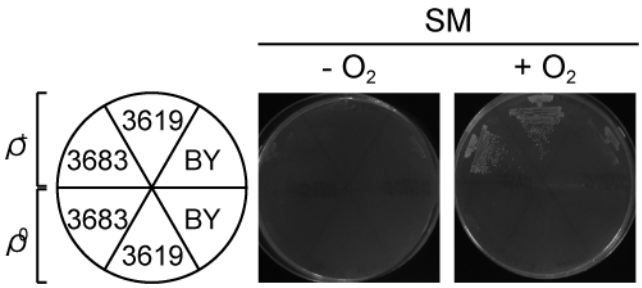

Fig. S1. BY4742, MK3619, and MK3683 were cultured on SM plates for 4 days in the absence ($-O_2$) or presence ($+O_2$) of oxygen. ρ^0 yeast strains, which are devoid of mitochondrial DNA, were created by growing cells in the presence of 25 μg/ml ethidium bromide (1). Anaerobic cultivation was performed using an AnaeroPack-Anaero (Mitsubishi Gas Chemical, Tokyo, Japan).

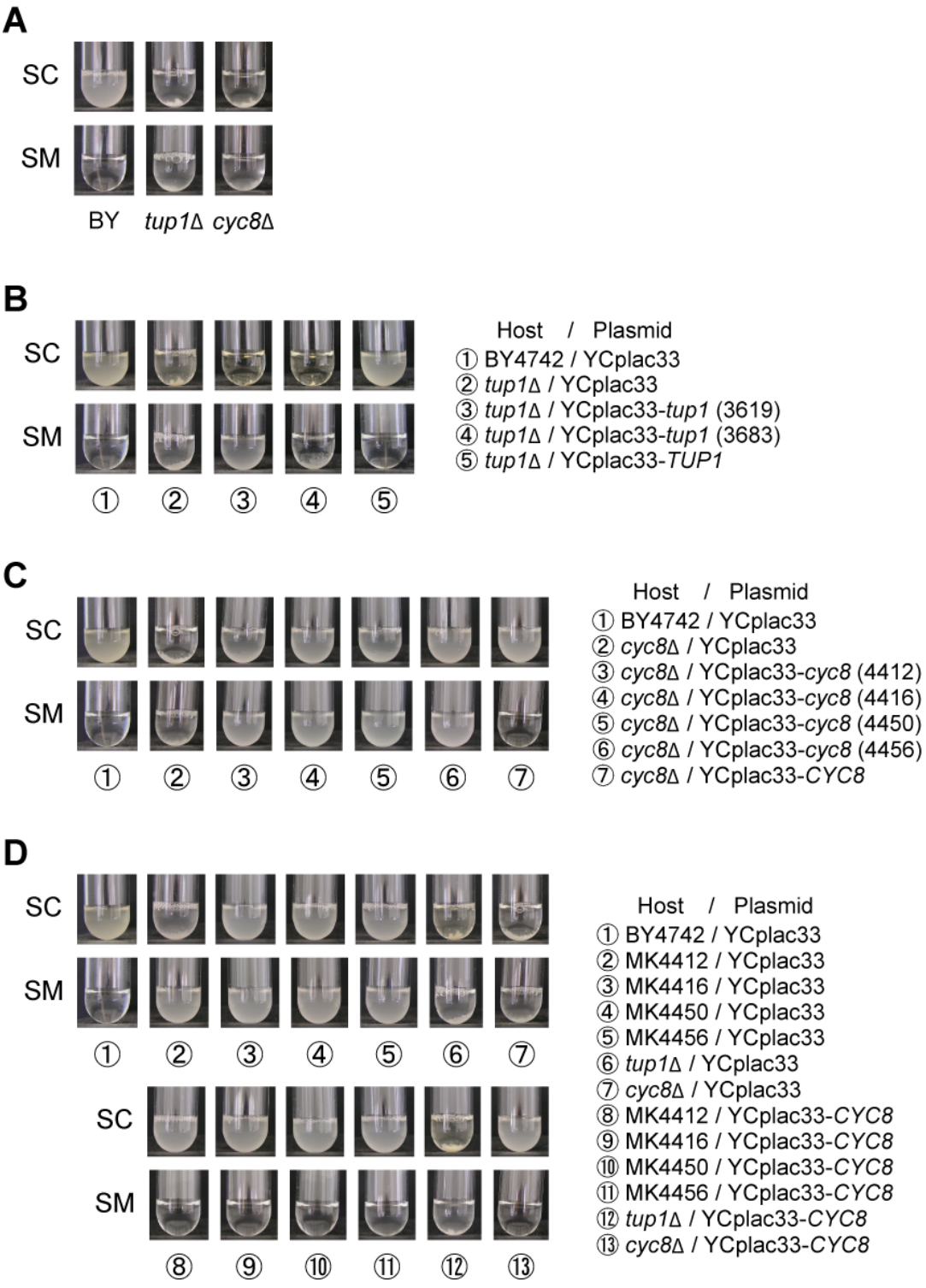

Fig. S2. Mutations in *TUP1* or *CYC8* are responsible for acquisition of mannitol assimilation and flocculation phenotypes. Representative images of the indicated strains cultured in SC or SM media, taken on day 3, are shown. (*A*) Culture images corresponding to Fig. 2A. (*B*) Culture images corresponding to Fig. 2C. (*C*) Culture images corresponding to Fig. 2D. (*D*) Culture images corresponding to Fig. 2E.

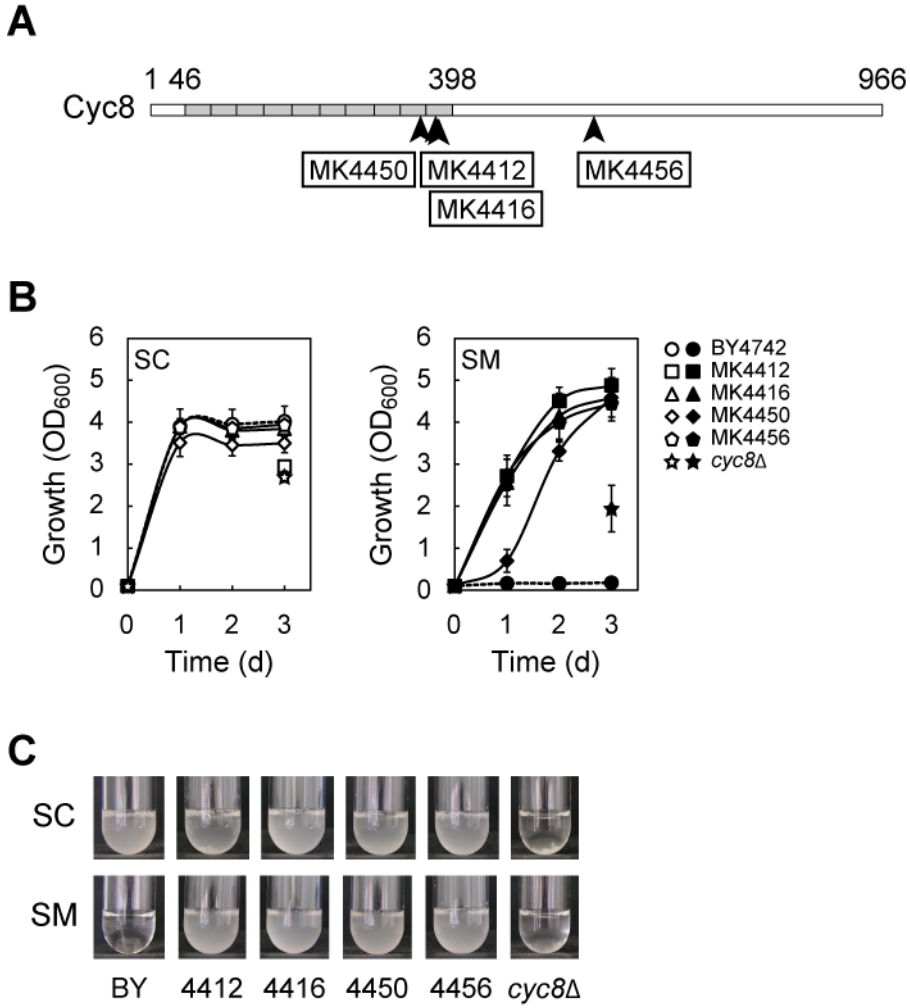

Fig. S3. Mutation in *CYC8* is responsible for acquisition of the ability to assimilate mannitol. (*A*) Schematic structure of Cyc8 (966 amino-acid residues). The TPR motifs are shaded (2). The mutated sites in MK4412, MK4416, MK4450, and MK4456 are indicated by arrowheads. (*B*) Growth of the indicated strains cultured in SC (open symbols) or SM (closed symbols) media. In the case of flocculated cells, growth was measured only on the third day. Results are the means of at least three independent experiments, and error bars represent SDs. (*C*) Representative images of the indicated strains cultured in SC or SM media, taken on day 3. Culture images correspond to Fig. S3B.

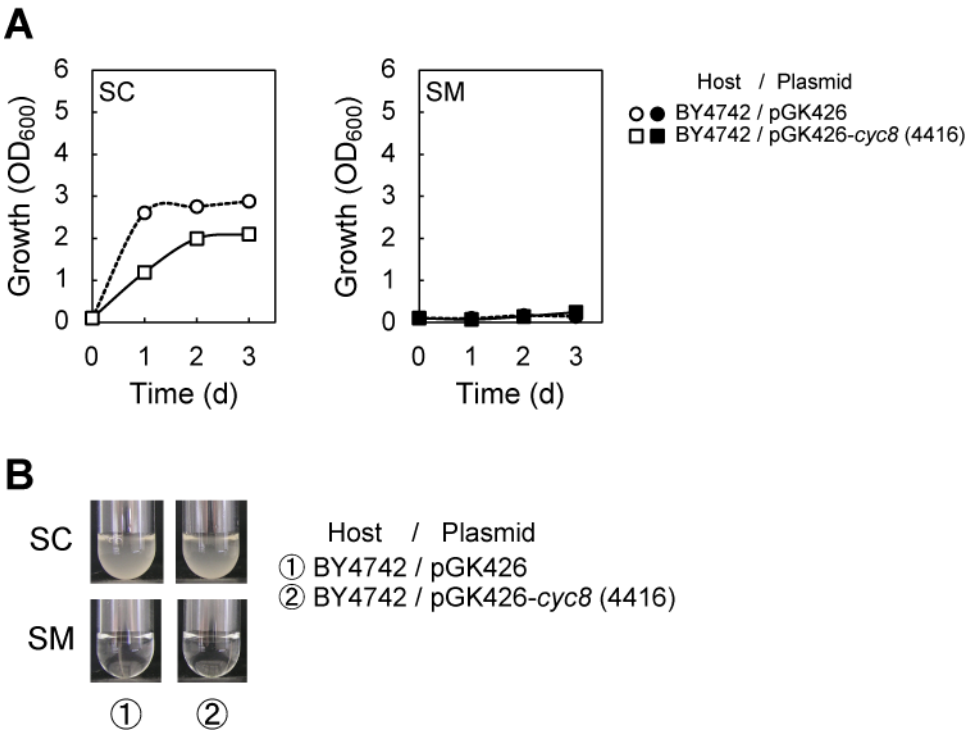

Fig. S4. Overexpression of the *CYC8* allele from MK4416 did not confer the ability to assimilate mannitol on wild-type *S. cerevisiae*. (*A*) Growth of the indicated strains cultured in SC (open symbols) or SM (closed symbols) media. Results are the means of three independent experiments, and error bars represent SDs. The *CYC8* allele from MK4416 cells was cloned into the high-copy plasmid pGK426 (3). The resultant plasmid was introduced into BY4742, and the *CYC8* allele was expressed under the control of the *PGK1* promoter. BY4742 carrying empty pGK426 was used as a control. (*B*) Representative images of the indicated strains cultured in SC or SM media, taken on day 3. Culture images correspond to Fig. S4A.

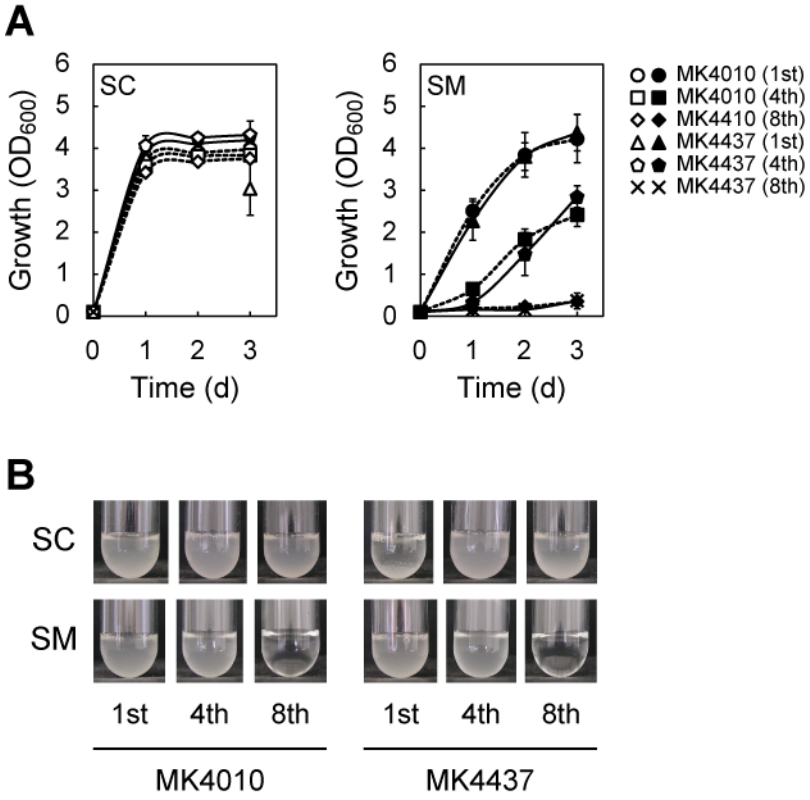

Fig. S5. MK4010 and MK4437 cells gradually lost the ability to assimilate mannitol during serial passage. (*A*) Growth of the indicated strains cultured in SC (open symbols) or SM (closed symbols) media. In the case of flocculated cells, growth was measured only on the third day. Results are the means of at least three independent experiments, and error bars represent SDs. Ordinal number in parentheses represents passage on YPG (for MK4010) and YPD (for MK 4437) plates. (*B*) Representative images of the indicated strains cultured in SC or SM media, taken on day 3. Culture images correspond to Fig. S5A.

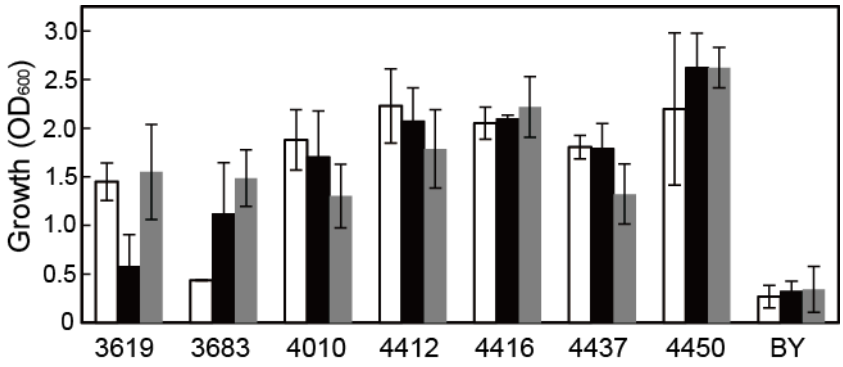

Fig. S6. Effects of pre-cultivation of Mtl+ strains in SC liquid medium on their growth in SM liquid medium. The indicated Mtl+ cells and BY4742 (BY), which were maintained on YPD plates (only MK4437) or YPG plates (the others), were inoculated to OD_{600} of 0.1 in 1.0 ml SM and SC liquid media in test tubes and grown at 30°C at 145 spm for 24 h. Then OD_{600} of the culture in SM medium was measured after addition of 0.1 ml 500 mM EDTA followed with vortexing, and is indicated by a white bar (pre-cultivation in SC medium, none). Cells in the SC medium were collected, washed three times with SDW, again inoculated to OD_{600} of roughly 0.1 in 1.0 ml SM and SC liquid media in test tubes, and grown as described above. Again, OD_{600} of the culture in SM medium was measured as above and is indicated by a black bar (pre-cultivation in SC medium, only once). Cells in SC medium were collected, washed, inoculated, and grown as described above. OD_{600} of the culture in SM medium was measured as described above and is indicated by a gray bar (pre-cultivation in SC medium, twice). Results are the means of three independent experiments, and error bars represent SDs.

Table S1. *S. cerevisiae* strains used in this study

Strain	Description	Source
AH109	*MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3 GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2 URA3::MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ MEL1*	Clontech
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	EUROSCARF
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	EUROSCARF
DBY877	*MATα his4-619*	Dr. Peter Novick
EBY100	*MATa leu2Δ1 ura3-52 his3Δ200 trp1 pep4::HIS2 prb1Δ1.6R can1 GAL GAL1-AGA1::URA3*	Invitrogen
SEY6210	*MATα leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 lys2-801suc2-Δ9*	Dr. Gabriele Fischer von Mollard
T8-1D	*MATα SUP11 ade2-1 mod5-1 ura3-1 lys2-1 leu2-3,112 his4-519*	Dr. Teresa Zoladek
YPH500	*MATα ura3-52 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ade2-101*	Dr. Teresa Zoladek
MK3619	Derived from BY4742	This study
MK3683	Derived from BY4742	This study
MK4010	Derived from BY4742	This study
MK4035	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 tup1Δ::kanMX4*	EUROSCARF
MK4410	Derived from BY4742	This study
MK4412	Derived from BY4742	This study
MK4416	Derived from BY4742	This study
MK4421	Derived from BY4742	This study
MK4437	Derived from BY4742	This study
MK4443	Derived from BY4742	This study
MK4446	Derived from BY4742	This study
MK4447	Derived from BY4742	This study
MK4449	Derived from BY4742	This study
MK4450	Derived from BY4742	This study
MK4456	Derived from BY4742	This study
MK4965	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 cyc8Δ::kanMX4*	EUROSCARF

Table S2. Genes that were upregulated by more than 4-fold in three conditions relative to the control (BY4742 cells grown in SC)

Systematic Name	Gene product	Fold change		
		3619 (SM)	3683 (SM)	3683 (SC)
YBR040W	Fig1, integral membrane protein required for efficient mating	17.14	4.14	5.65
YFL011W	Hxt10, putative hexose transporter	16.64	21.95	5.86
YEL070W[*]	Dsf1, putative mannitol dehydrogenase	14.20	16.39	8.63
YJR150C	Dan1, anaerobic cell wall mannoprotein	12.74	35.42	44.41
YER011W	Tir1, anaerobic cell wall mannoprotein	12.45	26.19	18.06
YML058W-A	Hug1, protein involved in the Mec1p-mediated checkpoint pathway	11.42	23.15	19.39
YOR348C	Put4, proline permease	11.30	7.62	4.82
YNL145W	Mfa2, mating pheromone a-factor	10.54	7.84	12.15
YOR028C	Cin5, Basic leucine zipper (bZIP) transcription factor of the yAP-1 family	9.59	8.70	4.46
YGR287C	Ima1, major isomaltase (alpha-1,6-glucosidase/alpha-methylglucosidase)	9.49	15.89	14.16
YOL157C	Ima2, isomaltase (alpha-1,6-glucosidase/alpha-methylglucosidase)	9.35	18.33	12.07
YIL172C	Ima3, alpha-glucosidase	8.46	17.02	11.62
YIL160C	Pot1, 3-ketoacyl-CoA thiolase with broad chain length specificity	8.21	6.23	4.90
YHR214C-E	Putative protein of unknown function	8.10	6.56	4.03
YDL244W	Thi13, protein involved in synthesis of the thiamine precursor HMP	7.47	8.36	5.27
YBR299W	Mal32, maltase (alpha-D-glucosidase)	6.64	7.91	6.59
YJR156C	Thi11, protein involved in synthesis of the thiamine precursor HMP	6.51	6.86	4.64
YIL015W	Bar1, aspartyl protease; helps cells find mating partners	6.46	5.58	9.18
YDL246C	Sor2, putative sorbitol dehydrogenase	6.45	10.42	5.12
YNL332W	Thi12, protein involved in synthesis of the thiamine precursor HMP	6.40	6.63	4.13

YGL089C	Mf(alpha)2, mating pheromone alpha-factor	6.27	5.97	4.52
YLR413W	Ina1, putative protein of unknown function	6.16	8.99	6.99
YFL058W	Thi5, protein involved in synthesis of the thiamine precursor HMP	5.96	6.19	4.07
YER096W	Shc1, sporulation-specific activator of Chs3p (chitin synthase III)	5.51	6.39	4.99
YDR461W	Mfa1, mating pheromone a-factor	4.24	4.21	6.22
YKR034W	Dal80, negative regulator of genes in multiple nitrogen degradation pathways	4.07	4.38	4.67

[*] *S. cerevisiae* has two putative homologs of mannitol dehydrogenase (*YEL070W* and *YNR073C*). *YEL070W* cannot be distinguished from *YNR073C* in microarray analysis because their nucleotide sequences are 98.9% identical.

Table S3. Putative hexose transporter genes* and their fold change in expression level relative to that of BY4742 cells in SC

Systematic Name	Gene product	Fold change		
		3619 (SM)	3683 (SM)	3683 (SC)
YHR094C	Hxt1, low-affinity glucose transporter of the major facilitator superfamily	0.11	0.29	0.87
YMR011W	Hxt2, high-affinity glucose transporter of the major facilitator superfamily	1.84	3.47	4.03
YDR345C	Hxt3, low affinity glucose transporter of the major facilitator superfamily	0.12	0.50	1.03
YHR092C	Hxt4, high-affinity glucose transporter; member of the major facilitator superfamily	0.11	0.38	1.33
YHR096C	Hxt5, hexose transporter with moderate affinity for glucose	2.03	1.66	0.76
YDR342C†	Hxt7, high-affinity glucose transporter; member of the major facilitator superfamily	0.92	0.87	1.25
YJL214W	Hxt8, protein of unknown function with similarity to hexose transporters	2.09	6.48	11.81
YJL219W	Hxt9, putative hexose transporter that has similarity to major facilitator superfamily (MFS) transporters	1.78	1.33	1.59
YFL011W	Hxt10, putative hexose transporter	16.64	21.95	5.86
YOL156W	Hxt11, putative hexose transporter that has similarity to major facilitator superfamily (MFS) transporters	1.58	1.52	1.54
YEL069C	Hxt13, hexose transporter	4.50	5.23	1.99
YNL318C	Hxt14, protein with similarity to hexose transporter family members	1.35	1.33	1.16
YDL245C‡	Hxt15, protein of unknown function with similarity to hexose transporters	2.16	2.57	1.48
YNR072W	Hxt17, hexose transporter	3.37	3.87	1.63
YLR081W	Gal2, galactose permease	2.26	1.45	1.55
YDL194W	Snf3, plasma membrane low glucose sensor that regulates glucose transport	1.46	1.20	0.96
YDL138W	Rgt2, plasma membrane high glucose sensor that	1.15	1.42	1.25

	regulates glucose transport

*Because *YIL170W+YIL171W* (Hxt12) is a possible pseudogene, it was not detected in microarray analysis.

†*YDR342C* (Hxt7) cannot be distinguished from *YDR343C* (Hxt6) in microarray analysis because their nucleotide sequences are 99.8% identical.

‡*YDL245C* (Hxt15) cannot be distinguished from *YJR158W* (Hxt16) in microarray analysis because their nucleotide sequences are 99.9% identical.

Table S4. 97 strains from the EUROSCARF haploid deletion sets* whose growth was examined in SM medium

adr1, *aft1*, *arl3*, *aro3*, *ash1*, *azf1*, *bas1*, *bem1*, *bio3*, *cin5*, *cup9*, *dan4*, *dot1*, *dot6*, *ecm22*, *eds1*, *fkh1*, *fkh2*, *flo1*, *flo8*, *flo10*, *gcn4*, *gcn5*, *gcr1*, *gis1*, *gts1*, *hap2*, *hap3*, *hap4*, *hap5*, *hda1*, *hda2*, *hek2*, *hhf1*, *hht1*, *hir1*, *hos2*, *hos3*, *ime2*, *isw2*, *med2*, *mga1*, *mig1*, *mig2*, *mot3*, *msn2*, *msn4*, *mss11*, *mtm1*, *muc1*, *nrg1*, *opi1*, *pgd1*, *phd1*, *pho4*, *put3*, *rco1*, *rfm1*, *rfx1*, *rgt1*, *rim101*, *rlm1*, *rox1*, *rpd3*, *rph1*, *rpn4*, *rtg1*, *rtg3*, *sap30*, *sdc1*, *set2*, *set3*, *shg1*, *sif2*, *sis2*, *skn7*, *sko1*, *sod1*, *sok2*, *srb5*, *ssn3*, *ssn8*, *stb5*, *sut1*, *swd1*, *swi4*, *swi5*, *tea1*, *tec1*, *tod6*, *ume1*, *upc2*, *whi2*, *xbp1*, *yap6*, *yer130c*, *zap1*

*Each gene was disrupted by the *kanMX4* marker. The genetic background of all strains was as follows: *MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*.

Supplemental References

1. **Fox TD, Folley LS, Mulero JJ, McMullin TW, Thorsness PE, Hedin LO, Costanzo MC**. 1991. Analysis and manipulation of yeast mitochondrial genes. Methods Enzymol. **194:**149-165.
2. **Sikorski RS, Boguski MS, Goebl M, Hieter P**. 1990. A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell **60:**307-317.
3. **Ishii J, Izawa K, Matsumura S, Wakamura K, Tanino T, Tanaka T, Ogino C, Fukuda H, Kondo A**. 2009. A simple and immediate method for simultaneously evaluating expression level and plasmid maintenance in yeast. J. Biochem. **145:**701-708.